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Studies of transcriptional changes induced by low dose ionizing radiation in three human skin models

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Evaluating the effects of low-dose ionizing radiation (LDIR) on humans is important for a number of reasons. First, there is still much unknown about the biological response and molecular mechanisms of human tissues exposed to LDIR. In the context of cancer treatment and radiation therapy it is imperative to define the potential effects of radiation that strikes outside the target treatment field on normal human tissue. Additionally, in the context of public policy, it is important to create a database upon which to base policy decisions on the limits of radiation exposure. We are investigating the molecular mechanisms of LDIR response in human skin using data from in vivo and ex vivo exposures, as well as exposure of an in vitro skin model. The in vivo human skin model consists of skin biopsies from consented patients undergoing radiotherapy. The ex vivo skin model consists of surgically excised human skin exposed to IR ex vivo. In comparison to the in vivo model, this model has the following advantages: 1) it facilitates frequent access to human skin samples, since they become available any time an abdominoplasty is performed on a consenting patient; and 2) it does not restrict sample size or experimental design since large pieces of skin are usually obtained. The in vitro skin model is EpiDermFT (MatTek, Ashland, MA), which is artificial skin made of normal human epidermal keratinocytes and normal human dermal fibroblasts grown in three dimensions. In comparison to real human skin, EpiDermFT offers the advantage of a well defined cell type composition.

Our present goal is to define characteristic gene expression profile changes in human skin exposed to LDIR. Irradiation was performed with an Elekta Synergy clinical irradiator (Stockholm, Sweden), at a dose rate of 4 Gy/min for in vivo and ex vivo irradiated skin, and of 0.5 Gy/min for EpiDermFT. Doses of X rays delivered were 0 and 0.1 Gy for in vivo irradiated skin, 0, 0.05 and 5 Gy for ex vivo irradiated skin, and 0, 0.1 and 1 Gy for EpiDermFT. Controls consisted of non-irradiated samples. Responses to exposure to single doses of LDIR were analyzed at the transcriptional level in all three human skin models using the Illumina (Illumina Inc., San Diego, CA) platform (Ref-8 Expression Beadchips with 24,535 RefSeq curated gene probes per microarray). Total RNA was extracted from control and/or test samples at 0, 3, 8 and 24 h post IR for in vivo and for EpiDerm FT, and 0, 2, 8 and 30 h post ex vivo irradiation of human skin. The software Metacore by Genego ® was used to map differentially expressed genes to functional pathways.

Analysis of the in vivo response of human skin to LDIR complements and follows two previous studies by our lab using similar techniques. The first report covered one time point over 3 LDIR doses – 0.01, 0.1, and 1 Gy, plus the non-irradiated control (Goldberg et al. 2006). The second report used one dose of 0.1 Gy and samples consisting of 3 mm punch biopsies and no duplicates were analyzed at 0, 3, 8 and 24 h post IR(Berglund et al. 2008). In this present study, 4mm punch biopsies were taken from 9 men, and all biopsies were split and treated as duplicates. Gene expression profiles of timepoints 3, 8, and 24 h were compared to the baseline (0 h). A total of 2009 genes were differentially expressed over all timepoints when considering FDR- corrected p values ≤ 0.05 as significant. At 3 h post-IR 528 genes were downregulated and 500 upregulated, at 8 h post-IR 410 genes were downregulated and 612 upregulated, and 485 genes were downregulated and 707 upregulated at 24 h post-IR. Apoptosis and survival pathways were upregulated at 3 and 24 h; developmental pathways were downregulated at 8 h; cell adhesion pathways were regulated at all three timepoints; and cell cycle pathways were upregulated at 3 and 8 h. Other pathways significantly affected (mostly at 8 h) were involved in DNA damage, immune response, signal transduction, cytoskeleton remodeling, and transcription.

Responses of human skin irradiated ex vivo were studied with RNA extracted from samples collected just before irradiation (T0), and 2, 8 and 30 h following exposure to 0, 0.05 and 5 Gy; duplicate samples from 2 patients were split and treated as quadruplicates. Genes were considered significantly differentially expressed if FDR-corrected p values were ≤ 0.01. Such genes increased in number with post-IR time for both doses. In response to 0.05 Gy, 4 genes were downregulated at 2 h post-IR; at 8 h post –IR 1 gene was upregulated, and at

30 h post-IR 115 and 196 were respectively down and upregulated. Some gene overlap between time points was identified at 5 Gy but none at 0.05 Gy; gene overlap between doses was observed only at 30h post-IR. At this same time, genes modulated by 0.05 Gy mapped to pathways involved in neurophysiological processes, development, and metabolism, while genes modulated by 5 Gy mapped to pathways involved in immune response, cytoskeleton remodeling, cell cycle regulation, transcription and development.

Responses of EpiDermFT to 0, 0.1 and 1 Gy were analyzed at 0, 3, 8 and 24 h post-IR. Genes were considered significantly differentially expressed if FDR-corrected *p* values were ≤ 0.05. In comparison to the controls, the numbers of genes significantly differentially expressed post-IR were as follows: at 3 h, 950 and 920 genes were modulated by 0.1 and 1 Gy respectively; at 8 h, 204 genes were responsive to 0.1 Gy and 906 to 1 Gy; and at 24 h 649 and 882 genes were modulated by 0.1 and 1 Gy respectively. At 3 h post-IR 455 genes were downregulated and 495 upregulated, at 8 h post-IR 227 genes were downregulated and 143 upregulated, and 378 genes were downregulated and 271 upregulated at 24 h post-IR. Genes responsive to 0.1 and 1 Gy mapped primarily to pathways involved in DNA damage, cell cycle, apoptosis and survival, immune response, development, transcription and cytoskeleton remodeling. At 3 h post-IR, the most prominent pathways for both IR doses were apoptosis and survival, cell cycle, transcription and immune response. DNA damage and cell cycle pathways were the most represented 8 h post-exposure to either 0.1 or 1 Gy, while pathways prevalent at 24 h post-IR were involved in cell cycle, development and cytoskeleton remodeling.

Comparisons between genes modulated in response to 0.05 or 0.1 Gy in all three human skin models show that they reach a maximum at 24 h post-IR in the in vivo and ex vivo irradiated skin models, while in EpiDermFT, this maximum is reached at 3 h post-IR. At early post-IR times, the overall cellular response, in all three skin models, is a downregulation of gene expression that is turned around at 24 h post-IR. Functional pathways involved in response to LDIR overlap in all three human skin models examined. This observation validates the use of the ex vivo and in vitro human skin models to study LDIR induced changes in the human skin proteome and metabolome.

References

Goldberg, Zelanna, David M. Rocke, Chad Schwietert, Susanne R. Berglund, Alison Santana, Angela Jones, Jörg Lehmann, Robin Stern, Ruixiao Lu, and Christine Hartmann Siantar (2006) "Human In Vivo Dose Response to Controlled, Low-Dose Low LET Ionizing Radiation Exposure," *Clinical Cancer Research*, **12**, 3723–3729.

Berglund, Susanne R., David M. Rocke, Jian Dai, Chad W. Schwietert, Alison Santana, Robin L. Stern, Joerg Lehmann, Christine L. Hartmann Siantar, and Zelanna Goldberg (2008) "Transient Genome-Wide Transcriptional Response to Low-Dose Ionizing Radiation In-Vivo in Humans," *International Journal of Radiation Oncology, Biology, Physics*, **70**, 229–234.